

Larval Host Use of the Fatmucket Mussel

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Abstract

In Minnesota, 52% of the state's mussel species are listed as Endangered, Threatened, or of Special Concern, and 6% have been extirpated. Recent population declines have urged immediate action to be taken before native mussels and their ecosystem services are lost forever. How successful we are at conserving a species depends, in part, on our knowledge of its life history needs. As particular fish species act as suitable hosts for the parasitic larvae of mussels, conservation efforts must include sustainable host fish management. A better understanding of the relationship that native *Lampsilis siliquoidea*, a rare species in parts of its range, has with fishes can assist natural resource managers in maintaining this mussel and its ecosystem services.

The purpose of this research was to expand our knowledge of the Fatmucket's (*L. siliquoidea*) breadth of host use by performing laboratory tests on previously untested fishes. Species from unstudied fish families, including Centrarchidae, Esocidae, Percidae, Cyprinidae, Catostomidae, and Gasterosteidae, were examined. Standard methods for identifying suitable Fatmucket hosts were followed and performed at the University of Minnesota. Gravid *L. siliquoidea* were collected in May 2021 from Rice Creek, Ramsey County, Minnesota, and held in the laboratory. The majority of fishes were collected from the 7-county metro area between May and July.

Of the 14 fish species inoculated with *L. siliquoidea* larvae, 7 facilitated larval development. The variety of fish species shown to be suitable hosts for the Fatmucket mussel suggests that additional hosts remain to be discovered. Conservation efforts will benefit from an improved understanding of the life history needs of *L. siliquoidea*, as well as the re-establishment of this species in areas where it has been extirpated.

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Introduction

Native mussels are keystone species in North America, for they provide both regulating and provisioning services (Freshwater Mollusk Conservation Society (FMCS) 2016). As these animals are generalized filter feeders, they increase the biotic integrity of freshwater ecosystems (FMCS 2016). Cilia along the gills of mussels facilitate the capture of algae, bacteria, protozoa, and other organic particles from the water column during respiration (Haag 2012). This filtered material is consumed by mussels and deposited in the forms of feces and pseudo-feces, which provide food for a variety of invertebrates and plants (Haag 2012). While pedal-feeding, or using a foot to feed upon or suspend seston, mussels aerate sediments and consequently lower water treatment costs (FMCS 2016). These animals also increase habitat heterogeneity by providing refugia for benthic algae, macroinvertebrates, and nesting fishes (FMCS 2016). Both living and dead mussel shells provide rich sources of calcium that support organisms across riverine and lacustrine food webs (Haag 2012). Since a loss of freshwater mussels and their services would likely lead to the destabilization of aquatic environments, it is critical for malacologists to guide conservation efforts (Strayer et al. 2004).

The Freshwater Mollusk Conservation Society recently published an article addressing one of the greatest conservation concerns in the United States, namely, the decline of freshwater mussels and their habitats (FMCS 2016). This concern is particularly evident in Minnesota, given that 52% of the state's native mussel species are listed as Endangered, Threatened, or of Special Concern, and 6% have been extirpated (Minnesota Department of Natural Resources (MN DNR) 2020). For instance, *Lampsilis siliquoidea*, once a widespread species in Minnesota (Sietman 2003), is now nearly extirpated from the Blue Earth River basin (MN DNR 2020). Mussel populations are in decline due to habitat loss, degraded water quality, land-use changes,

overharvesting, and exotic species introductions (Strayer et al. 2004). Additionally, changes in fish communities and distribution also affect sustainable mussel recruitment. Hove et al. (2011) recommend that conservation efforts include sustainable host fish management. Therefore, a better understanding of the relationships that mussels have with fish species can help maintain these animals and their ecological benefits.

As early as the Cretaceous period, mussel fauna has been coevolving with fish fauna on a phylogenetic basis (Haag 2012). Fish dispersal primarily influences the abundance and diversity of freshwater mussels (Haag 2012). Some fishes in North America have developed immunological compatibility with the parasitic larvae (glochidia) of certain mussel species, acting as either their primary or marginal hosts (Haag 2012). Therefore, host competition among mussels has been a strong selective force in their speciation and evolution of host infection strategies (Haag 2012). Like most freshwater mussels, female *L. siliquoidea* display ornate mantle flaps to elicit attacks from potential hosts (Haag 2012). These mantle flaps have evolved to resemble small fishes that lure larger fishes to strike the soft tissue (Haag 2012). Upon rupture of the mantle margin, glochidia are released from nearby gill chambers (Haag 2012). Rows of micropoints on a glochidium allow it to attach to the gills, opercula, and fins of a suitable host species (Haag 2012). Glochidia derive their nutrients from the fish until metamorphosis is complete; juveniles detach from the host and burrow into the substrate where their life cycles will continue (Haag 2012).

It appears that the Fatmucket mussel has broad immunological compatibility with fishes in North America (Haag 2012). Based on previous findings, certain fish species from the families Centrarchidae, Percidae, Lepisosteidae, Ictaluridae, Moronidae, Cyprinidae, Peociliidae, and Gobiidae act as suitable hosts for the Fatmucket's larval development (Watters et al. 2009).

However, current research exhibits variability in host suitability within and between families (INHS Freshwater Mussel Host Database 2020). Species from unstudied fish families, including Gasterosteidae, Cyprinidae, Catostomidae, and Esocidae, have yet to be tested for host suitability. Although many species of Centrarchids are known hosts (Watters et al. 2009), two common fishes, *Lepomis gulosus* and *L. humilis*, require further inspection. Species from the family Percidae, particularly the darter species (*Etheostoma* spp. and *Percina* spp.), are also in question. Therefore, the purpose of this research was to enhance our understanding of the Fatmucket's breadth of host use by performing laboratory tests on previously untested fish species from Minnesota.

Methods

A three-phase plan was implemented to gather more information on the host use of *Lampsilis siliquoidea*. Phases 1b - 3 were repeated several times over the course of four months.

Phase 1a: Collect Fatmucket mussels; Phase 1b: Collect untested fish species.

Phase 2: Conduct laboratory host suitability trials.

Phase 3: Examine tank water for Fatmucket juveniles under a dissecting microscope.

Phase 1a: Collect Fatmucket mussels. Four female Fatmucket mussels were acquired from Rice Creek, Ramsey County, to provide glochidia for the host suitability trials. These animals were collected during May 2021 when females naturally start releasing their glochidia (Haag 2012). The mussels were transported to the University of Minnesota Wet Laboratory (UMN), where they were kept in an aquarium. Water temperature was held between 19-21 °C. They were returned to Rice Creek after all host suitability trials had been completed.

Phase 1b: Collect untested fish species. The collection of fishes began in May and ended on the first of July. The majority of fishes were collected from bodies of water within the

7-county metro area and kept in aquaria at the UMN. Fishing techniques, such as electrofishing, sieving, and dip-netting, were used to collect fishes for this study. It was assumed that at least some fishes would have previous exposure to *L. siliquoidea* glochidia (Dodd et al. 2005).

Therefore, to sterilize the gills before inoculation, the fishes were held in aquaria for at least three weeks prior to Phase 2. Water temperatures were held between 19-21 °C for most trials, but some fishes were held between 11-21 °C, due to an unplanned water heater outage.

Phase 2: Conduct laboratory host suitability trials. Standard methods for host suitability trials (Hove et al. 2016) were followed and performed at the UMN. Glochidia were retrieved by prying open the shells approximately 1 cm, using a surgical knife to cut open the gill marsupia, and rinsing out the larvae with water. Four separate beakers were used to collect glochidia from each mussel. After retrieval, the health of the glochidia was tested via salt exposure. About 100 glochidia from each beaker were pipetted into separate Petri dishes and examined under a dissecting microscope. In this study, at least 75% of the examined glochidia became agitated from salt penetration, so all glochidia were considered viable, and the remaining glochidia were used for fish inoculations.

Fishes were inoculated with glochidia to determine the suitable hosts of *L. siliquoidea*. Fishes were held in aquaria for at least 3 weeks to allow any natural glochidia infestations to be released. After this time, the fishes were placed into heavily aerated containers and inoculated with Fatmucket larvae for about 20 seconds. Most trials were conducted between 19-21 °C, although the temperatures of some aquaria were lowered to between 11-16 °C, due to unplanned water heater maintenance. After glochidia exposure, fishes were placed into clean water to await inspection. A single fish from each species was inspected under a dissecting microscope. All members of a species were re-inoculated if the fish's gills had not been infested with at least 15

glochidia. Once inoculated, fishes were moved into species-specific aquaria until the end of Phase 3.

Phase 3: Examine tank water for Fatmucket juveniles under a dissecting microscope. Water from the aquaria was studied to determine if glochidia metamorphosis had occurred. Outflowing water from each species-specific aquarium was filtered through a sieve (150 μm), and the solid residue was rinsed into a Petri dish. Material from the bottom of larger aquaria was siphoned using a hose and filtered through sieves (1 mm and 150 μm). Residue was collected bi-weekly and studied under dissecting microscopes for the presence of glochidia or juvenile mussels. Counts of glochidia and juvenile mussels were made and recorded for each fish species. The number of deceased fishes, as well as the number of glochidia on the gills of each deceased fish, were recorded during these processes. Deceased fishes were properly disposed of at the UMN. Once a fish species stopped releasing glochidia or juvenile mussels, the fishes were euthanized and disposed of following UMN IACUC protocol. Fish and mussel nomenclature follow Williams et al. (2017) and Page et al. (2013), respectively.

Results

Potential Fatmucket hosts were determined based on data collected from the host suitability trials. Species that facilitated the metamorphosis of *L. siliquoidea* glochidia were deemed suitable hosts. Juvenile mussels were recovered from these fishes. Of the 14 fish species infested with Fatmucket larvae, 7 facilitated glochidia metamorphosis (Table 1). Suitable host studies for the Black Crappie and Orangespotted Sunfish were still underway at the writing of this paper. The number of juveniles released by potential hosts ranged from 3 to 447. Of the remaining 7 fish species inoculated with *L. siliquoidea* glochidia, none facilitated metamorphosis.

Table 1. *Lampsilis siliquoidea* host suitability trial results. * Some fishes were held between 11-16 °C after day 33, due to an unplanned water cooling event. † Denotes newly identified suitable host species.

Fish species	Trial	Number inoculated	Number of survivors	Number of juveniles recovered	Juvenile release/glochidia attachment period (d)**
Common Shiner (<i>Luxilus cornutus</i>)	1	1	1	0	6-9
Bullhead Minnow (<i>Pimephales vigilax</i>)	1*	10	5	0	4-8
Blacknose Dace (<i>Rhinichthys atratulus</i>)	1	2	2	0	6-9
Creek Chub (<i>Semotilus atromaculatus</i>)	1*	1	1	0	4-8
White Sucker (<i>Catostomus commersonii</i>)	1	3	2	0	20-23
Northern Pike (<i>Esox lucius</i>) †	1	3	2	447	20-30
Warmouth (<i>Lepomis gulosus</i>)	1 2*	5 5	5 3	0 0	17-20 18-22
Orangespotted Sunfish (<i>Lepomis humilis</i>) †	1*	CONT	CONT	3?	25-36 (Trial still running)
Bluegill (<i>Lepomis macrochirus</i>)	1	13	13	55	17-24
Black Crappie (<i>Pomoxis nigromaculatus</i>)	1*	1	1	15?	32-36 (Trial still running)
Iowa Darter (<i>Etheostoma exile</i>) †	1	17	17	105	20-30
Johnny Darter (<i>Etheostoma nigrum</i>) †	1	10	5	6	16-20
Blackside Darter (<i>Percina maculata</i>)	1*	3	2	0	8-11

Slenderhead Darter (<i>Percina phoxocephala</i>) †	1	8	8	6	13-16
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**Range of days juvenile mussels or glochidia were recovered.

Discussion

The host suitability trial results are generally consistent with previous research. Past research has identified a variety of hosts for Fatmucket glochidia (see review in Watters et al. 2009). The observations of Bluegill and Black Crappie serving as suitable Fatmucket hosts are consistent with several studies (Coker et al. 1921, Keller and Ruessler 1997). The observations of Iowa Darter and Johnny Darter acting as suitable Fatmucket hosts are inconsistent with previous research, involving four *Etheostoma* species from the family Percidae (O'Dee and Watters 2000). It was observed that the Northern Pike and Orangespotted Sunfish facilitated glochidia metamorphosis, although these species did not serve as potential hosts in Keller and Ruessler (1997), and Howard et al. (1923), respectively. Additionally, this study discovered that the Slenderhead Darter is a suitable host species for *L. siliquoidea*.

The variety of fishes shown to be suitable hosts for *L. siliquoidea* suggest that additional potential hosts remain to be discovered among Centrarchids. Although the Fatmucket mussel has potentially widespread host use, the number of fish species tested in this study was limited to project funds. Several host suitability trials had not been fully completed due to the death of fishes, e.g. Bullhead Minnow, White Sucker, Northern Pike, Johnny Darter, Warmouth, and Blackside Darter. In the future, fishes will need to be more closely monitored, during Phase 2, to prevent over-infestation of the gills. It appears that inoculation loads should be less than 20 glochidia/fish for small fishes, and less than 100 glochidia/fish for larger fishes. The amount of time needed to inoculate fishes with glochidia varies among species.

This study revealed that *L. siliquoidea* is immunologically compatible with several previously untested fish species. Conservation efforts will benefit from an improved understanding of the early life history needs of the Fatmucket mussel in Minnesota. Proper management of *L. siliquoidea* involves ensuring that its hosts are at least present in its environment. Therefore, mussel hatchery managers can use newly identified hosts to propagate juveniles for the augmentation of diminished Fatmucket populations, or the re-establishment of this species in areas where it has been extirpated.

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